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Improvements in the decapsulation technique of *Artemia* cysts

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Abstract

Because bleaching powder is the cheapest source of hypochlorite in many countries of the world, a decapsulation procedure has been worked out in which technical calciumhypochlorite $Ca(OCl)_2$ is utilized.

A standard procedure has been developed for the deactivation of the chlorine residues adsorbed on the decapsulated cysts. Proper dehydration and storage of the decapsulated cysts assure maximum viability of the embryos in diapauze. A protoype system for routine decapsulation and processing of large quantities of cysts is proposed.

Beneficial effects of the decapsulation process on both the hatching percentage and the individual dry weight of the hatched nauplii are discussed in detail.

Introduction

As a result of its beneficial effects on the use of brine shrimp nauplii in aquaculture hatcheries, the decapsulation of *Artemia* cysts (Sorgeloos *et al.*, 1977 ; Bruggeman *et al.*, 1979) is practised more and more. Through the feedback information received from people who apply our methods, we have been able to identify some aspects that needed more study for the further improvement of cyst decapsulation. In this regard, attention was paid to the use of a cheaper source of hypochlorite, a better method to deactivate chlorine residues was developed, and a new system for more simplified routine decapsulation has been worked out. The beneficial effects of the decapsulation treatment on the hatching percentage and the individual dry weight of the hatched nauplii is reported for nine geographical strains of brine shrimp.

The use of calciumhypochlorite

In many countries of the world calciumhypochlorite $Ca(OCl)_2$, also called bleaching powder or chloride of lime, is a cheaper source of active chlorine than liquid bleach NaOCl. It

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can be found in various technical grades and is mostly used for desinfection purposes *e.g.* in swimming pools or culture tanks in aquaculture hatcheries. It is a much more stable product than liquid bleach and can be stored for longer periods. Furthermore its activity is usually correctly mentioned on the label of the commercial products (mostly 70% weight percent activity).

The ratios of cysts to active product (2 g to 1 g) and cysts to volume of decapsulation solution (15 g/200 ml) are identical to the values reported earlier for the treatment with NaOCl (Bruggeman *et al.*, 1979). Neither NaOH nor $Ca(OH)_2$ may be used to stabilize the pH of the decapsulation solution since they interact with $Ca(OCl)_2$ and drastically lower the hatchability of the decapsulated cysts. Optimal results are obtained with 50 g Na_2CO_3 or 30 g CaO/l decapsulation solution ; Tunsutapanich (personal communication) recommends the latter product because of its cheaper price. Since a precipitate is formed in the decapsulation solution, only the supernatant should be used in order to avoid clogging of screens when the treated cysts are washed.

For routine decapsulations we advise the following stepwise procedure : first dissolve the bleaching powder (aerate 10 min) ; add the technical CaO or Na_2CO_3 (aerate another 10 min) ; store overnight for precipitation (and eventually cooling off) ; the next morning siphon the supernatant off and use for decapsulation.

Processing and storage of decapsulated cysts

In a previous paper (Bruggeman *et al.*, 1979) we have described a sodiumthiosulphate treatment for the deactivation of the chlorine residues that remain adsorbed on the decapsulated cysts even after thorough washing with tap water. This method is, however, not entirely statisfactory because upon long-term storage of decapsulated cysts at high densities, the hatchability decreases. A tentative explanation is that the pH at which the cysts are decapsulated, leads to the formation of a saponification layer around the embryos, into which chlorinated compounds are trapped. Apparently the latter are not entirely deactivated by the thiosulphate.

The technique used in the chlorine treatment of plant seeds (Abdul-Baki, 1974) proved to be applicable for brine shrimp cysts : after washing out the hypochlorite, the cysts are treated with a 0.1 N HCl or HAc solution and are then thoroughly washed with tap water. This treatment ensures a higher viability upon storage in brine than the previous manipulation with thiosulphate.

As reported earlier, the storage of decapsulated cysts in saturated brine solution turns out to be a handy technique. Since the preparation of saturated brine is not a very easy task, the use of the Sterling Brinomat® (Spotte, 1970 ; Fig. 1), which can be assembled with very simple materials available in most aquaculture hatcheries, is highly recommended. With this simple apparatus saturated and at the same time filtered brine is made up continuously and automatically by gravity flow of tap water through packed layers of bulk salt.

The storage of decapsulated cysts in brine solutions has nonetheless its limitations. During the first months after decapsulation the cysts keep their maximum hatchability, even when stored at room temperature (± 20 °C). Over longer periods, cyst viability appears, however, to decrease as a function of storage temperature : *e.g.* after 6 months storage at room temperature the hatching efficiency dropped to below 50% whereas after 2 years storage at -4 °C, the

hatching percentage was still about 70%. According to Clegg (personal communication) the decreased hatchability of decapsulated cysts stored in brine is probably due to their relatively high water content (about 20%). When the water content ranges from 10 to 35%, indications of enzyme activity and a slow but significant drop in the ATP concentration have been reported by Clegg and Cavagnaro (1976). We have observed that in function of storage time and temperature, more and more cysts which, after dehydration, have a typical coffee-bean structure, turn opaque and become spherical. Initially these opaque cysts hatch faster than their coffee-bean shaped homologs ; however, after a longer exposure to the brine, they loose their viability.

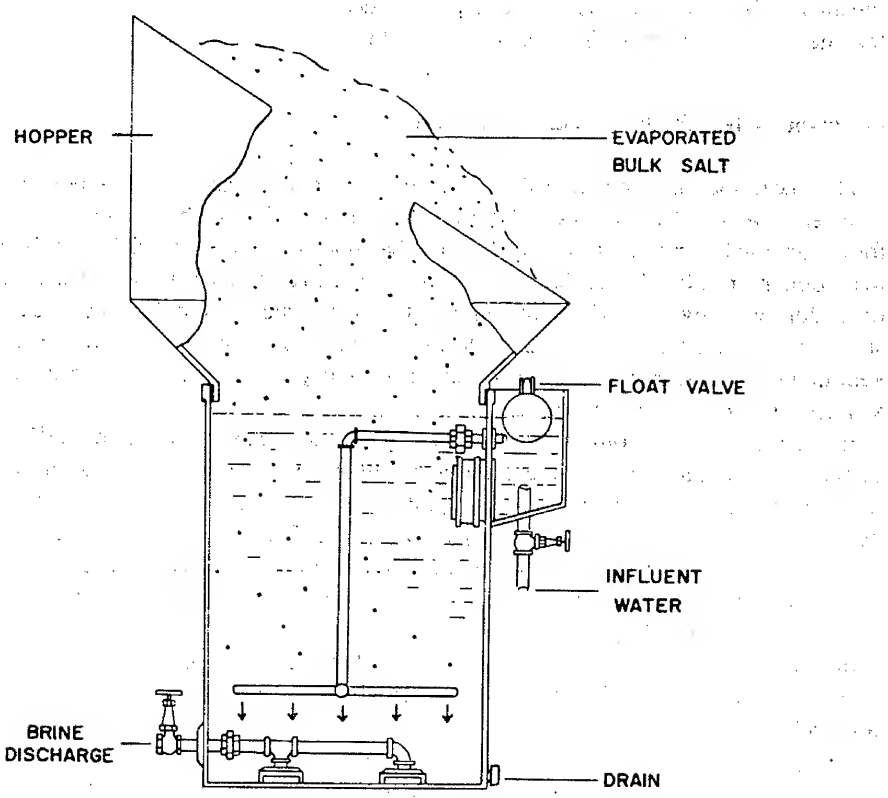

FIG. 1. Schematic diagram of the Sterling Brinomat® (after Spotte, 1970).

The metabolic processes which occur at a 20% water content are probably delayed to a large extent when the cysts are stored at freezing temperatures : *e.g.* after 1 year storage in brine, the hatching efficiency of untreated cysts had dropped to ±65% for two samples stored at respectively 25 °C and 4 °C but only to ±75% for a sample stored at -20 °C (Sorgeloos, 1979). Kinne (1977) and Richard (Salins du Midi-France, personal communication) have found that following low temperature storage (-20 to -30 °C) cysts should be

exposed to room temperature for 1 week minimum, prior to incubation, in order to avoid poor hatching yields. We have not yet observed this phenomenon which might be strain specific.

Long-term storage of dried decapsulated cysts (water content below 5%) is possible when they are kept in a dry and oxygen-free medium (nitrogen flushed or vacuum sealed containers). Air or freeze-drying can be successfully applied – without affecting the hatching efficiency – with decapsulated cysts which, upon dehydration in saturated brine, have been quickly washed free of salt with cold water.

Although we have not studied the phenomenon in detail yet, the major problem which remains to be solved in large scale drying of decapsulated cysts, is the persistent sticking together of these cysts when an air-drying technique is used.

Prototype system for large scale decapsulation

The decapsulation procedure described in our previous papers (Sorgeloos *et al.*, 1977 ; Bruggeman *et al.*, 1979) works well for small quantities. The method is not handy, however, for processing kilograms of cysts and cannot be applied on a commercial scale. In an attempt to automatize routine decapsulation we have developed an alternative for the manual addition of ice during the hypochlorite treatment and for the repeated manipulation of the cysts at each step in the decapsulation procedure. During the entire decapsulation treatment the cysts are kept in a cylindro-conical tank (see schematic drawings in Fig. 2 and 3), completely made of stainless steel mesh (150 μm mesh size).

In order to optimize both the circulation of the cysts within the container and the exchange of the internal solution with the external medium, the tank is equiped with two separate aeration systems : the first is a tube extending to the bottom of the funnel, the second is a perforated tube installed in the lower part of the cylinder acting as an aeration collar.

The only manual work during the decapsulation treatment consists of the consecutive transfers of the decapsulation container to the next bath (see scheme in Fig. 4) in the following sequence : seawater, hypochlorite, tap water, chloric acid, tap water and finally saturated brine. In the decapsulation step, the hypochlorite is kept at a temperature below 35 °C by continuous circulation through a cooling element (a cupper coil submerged in a bath of salt and ice).

The prototype container shown in Fig. 3 is dimensioned to treat 1 kg of cysts. Large scale experiments should now be considered to test the applicability of this system at a commercial scale.

Beneficial effects of the decapsulation treatment

We have reported earlier that the decapsulation technique has at least three major advantages for the use of *Artemia* in aquaculture hatcheries, namely desinfection of the *Artemia*, superfluity of separation of cyst shells from the hatched nauplii, and last but not least the potential use of decapsulated cysts as a direct food source for fish and crustacean larvae (Sorgeloos *et al.*, 1977).

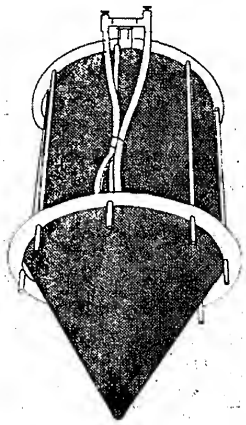

FIG. 2. Side view of the decapsulation container.

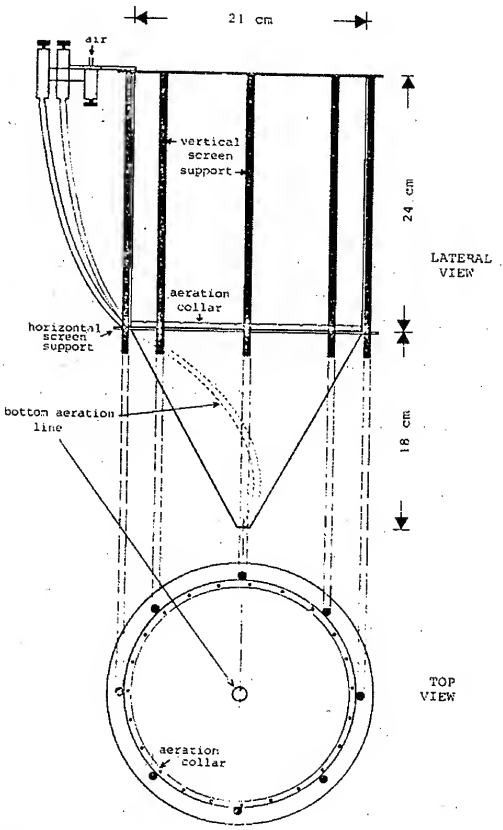

FIG. 3. Lateral and top view of the decapsulation container.

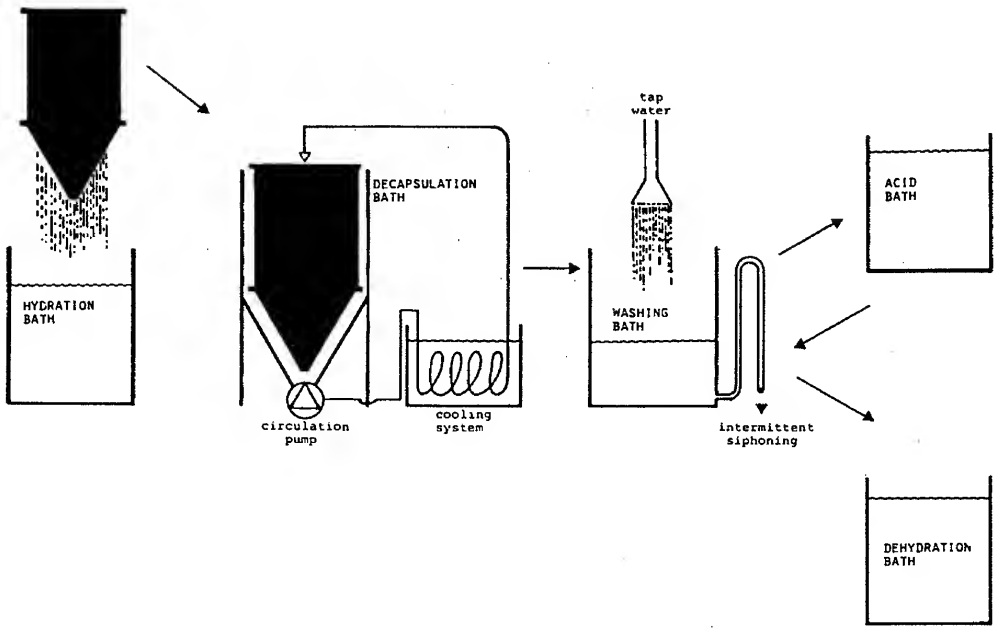

FIG. 4. Schematic diagram of the consecutive steps in the decapsulation procedure.

With regard to the latter aspect recent culture tests have demonstrated that decapsulated cysts are at least as good a food as freshly hatched nauplii for the larvae of *Metapenaeus monoceros* (Royan, 1980); *Penaeus monodon*, *Penaeus indicus*, *Metapenaeus ensis*, *Metapenaeus endevouri* and *Macrobrachium rosenbergii* (Laviña and Figuerosa, 1980), *Scylla serrata* and *Portunus pelagicus* (A. Laviña, personal communication).

Good growth and survival rates were reported when feeding decapsulated *Artemia* cysts to larvae of *Chanos chanos* (de los Santos *et al.*, 1980) and *Lebistes reticulatus*, *Xiphonophorus helleri* and *X. maculatus* (Stephanou, personal communication).

Departing from an earlier observation that for some strains the hatchability of the cysts increased upon decapsulation (Sorgeloos, 1979), we started a detailed study on this particular subject with cysts from nine geographical origins (Table I). The test material was first processed and cleaned following the procedure described in Sorgeloos *et al.* (1978). The following aspects were studied on untreated and decapsulated cysts:

a) the hatching percentage: 10 replicates of about 100 cysts each were hatched out in glass petri dishes at 25 °C in 35 ‰ seawater under continuous illumination;
b) the individual dry weight of instar I nauplii, measured according to the methodology described by Vanhaecke and Sorgeloos (1980);
c) the total weight of nauplii obtained from 1 g of untreated cysts.

The data obtained are summarized in Tables I, II and III.

For most strains both the hatching percentage and the individual dry weight of the nauplii increase significantly when the cysts have been decapsulated. This can eventually be explained

TABLE I

Hatching percentage (mean $\bar{x}$, and standard deviation s) at 25 °C, in 35 ‰ seawater, of untreated and decapsulated cysts from various geographical origin

Origin of cysts	Untreated cysts		Decapsulated cysts	
	$\bar{x}$	s	$\bar{x}$	s
San Francisco Bay, California, USA (San Francisco Bay Brand,Inc., batch 288-2596)	71.4	4.5	82.1*	7.9
Macau, Brazil (Companhia Industrial do Rio Grande do Norte, batch 871172)	82.0	9.0	91.5*	2.0
Great Salt Lake, Utah, USA (harvest 1977)	43.9	5.2	54.6*	8.0
Shark Bay, Australia (World Ocean Pty, batch 114)	87.5	4.8	90.9	2.4
Buenos Aires, Argentina	62.8	3.9	84.5*	5.0
Galera Zamba, Colombia	80.4	9.3	91.6*	4.1
Margherita di Savoia, Italy (harvest 1977)	77.2	4.7	84.8*	2.9
Chaplin Lake, Canada	11.9	3.0	39.3*	8.6
San Pablo Bay, California, USA (San Francisco Bay Brand, Inc., batch 1628)	84.3	5.2	90.8*	1.2

* Significant increase at the 0.05 level of the hatching percentage.

by the fact that the resistance and the strength of the outer cuticular membrane are reduced by removal of the chorion, respectively the oxidative effect of the hypochlorite. If this is the case, breaking becomes possible at a lower glycerol concentration than that necessary for untreated cysts. The "trehalose-glycerol hyperosmotic regulatory system" – theory of Clegg (1964) and Conte *et al.* (1977) which we already used to explain the increased hatchability and the higher naupliar dry weight of cysts hatched at low salinity (Sorgeloos, 1980), could thus also be involved here to explain the better results with decapsulated cysts. The differences in the energy reserves of individual cysts within a particular strain (or cyst batch) might be so important, that the individual dry weight of nauplii that can not hatch out from untreated cysts, but only from decapsulated cysts, is much lower than that of the controls. This might explain the rather small differences which were noted in some cases between the two series for the average individual dry weight of the nauplii.

An immediate practical extrapolation of the previous finding is that by using decapsulated cysts in aquaculture hatcheries, the fish or crustacean species cultured have a "bigger" *Artemia* nauplius at their disposal which could result in a lower energy expenditure for their food uptake. In this regard it also becomes interesting to analyze the biochemical composition of the naupliar weight gain resulting from decapsulation.

Last but not least, it is clear from Table III that in function of the *Artemia* strain used, application of the decapsulation technique can lead to a substantial economy of the quantity of cysts needed for aquacultural purposes.

TABLE II

Individual dry weight (in μg, mean $\bar{x}$, and standard deviation s) of instar I nauplii hatched out at 25 °C in 35 ‰ seawater, of untreated and decapsulated cysts from various geographical origin

Origin of cysts	Untreated cysts		Decapsulated cysts		Procentual difference
	$\bar{x}$	s	$\bar{x}$	s	
San Francisco Bay, California, USA (San Francisco Bay Brand, Inc., batch 288-2596)	1.63	0.11	1.74	0.09	+ 6.7
Macau, Brazil (Companhia Industrial do Rio Grande do Norte, batch 871172)	1.74	0.08	1.78	0.05	+ 2.3
Great Salt Lake, Utah, USA (harvest 1977)	2.41	0.11	2.36	0.12	− 2.1
Shark Bay, Australia (World Ocean Pty, batch 114)	2.47	0.13	2.61	0.15	+ 5.7
Buenos Aires, Argentina	1.72	0.07	1.90*	0.10	+ 10.5
Galera Zamba, Colombia	2.27	0.08	2.26	0.15	− 0.4
Margherita di Savoia, Italy (harvest 1977)	3.33	0.18	3.60*	0.23	+ 8.1
Chaplin Lake, Canada	1.97	0.13	2.05	0.09	+ 4.1
San Pablo Bay, California, USA (San Francisco Bay Brand, Inc., batch 1628)	1.92	0.08	1.99	0.05	+ 3.6

* Significant increase at the 0.05 level of the hatching efficiency.

TABLE III

Naupliar biomass (in mg dry weight) obtained out of 1 g cyst material, untreated, respectively decapsulated, hatched at 25 °C in 35 ‰ seawater

Origin of cysts	Untreated cysts	Decapsulated cysts	Procentual difference
San Francisco Bay, California, USA (San Francisco Bay Brand, Inc., batch 288-2596)	435.5	534.6	22.8
Macau, Brazil (Companhia Industrial do Rio Grande do Norte, batch 871172)	529.0	603.8	14.1
Great Salt Lake, Utah, USA (harvest 1977)	256.5	311.1	21.3
Shark Bay, Australia (World Ocean Pty, batch 114)	537.5	590.0	9.8
Buenos Aires, Argentina	333.0	494.9	48.6
Galera Zamba, Colombia	185.2	210.1	13.4
Margherita di Savoia, Italy (harvest 1977)	458.2	544.1	18.7
Chaplin Lake, Canada	15.8	54.2	243.0
San Pablo Bay, California, USA (San Francisco Bay Brand, Inc., batch 1628)	497.7	555.6	11.6

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